Genetic mapping of HA-R4 identified downy mildew resistance gene cluster to races 300, 770, and 734

Zhao Liu, Thomas J. Gulya, Gerald J. Seiler,
Brady A. Vick, and C. C. Jan

NDSU NORTH DAKOTA STATE UNIVERSITY

USDA ARS

Introduction

- Downy mildew is one of the main diseases of cultivated sunflower (*Helianthus annuus* L.), caused by *Plasmopara halstedii* (Farl.) Berl. & de Toni. It attacks the root, cotyledon and true leaves of sunflower and is transmitted through soil, air, and sunflower plants, resulting in up to 80% loss of yield.
- The major dominant resistance genes to this disease have been designated as *Pl* genes. Up to now, more than 21 resistance genes have been reported ($Pl_{1\text{-}13}$, *Plv*, *Plw*, *Plx-z*, *Mw*, *Mx*, and Pl_{Arg}).
- Eight *Pl* genes have been mapped to linkage maps using molecular markers, such as the restriction fragment length polymorphism (RFLP), simple sequence repeats (SSR), sequence tagged sites (STS), and random amplified polymorphic DNA (RAPD), etc.

Pl genes and clusters mapped in sunflower

Genes	Linkage group	Reference
Pl_1, Pl_2, Pl_6, Pl_7	Gedil et al. 2001, RFLP LG 1 Yu et al. 2003, SSR LG 8	Mouzeyar et al. 1995; Roeckel-Drevet et al. 1996; Vear et al. 1997; Gedil et al. 2001; Bouzidi et al. 2002; Pankovic et al. 2007; Radwan et al. 2008
Pl_5, Pl_8	Gentzbittel et al. 1999, RFLP LG 6; Yu et al. 2003, SSR LG 13	Bert et al. 2002; Radwan et al. 2003, 2004, 2008
Pl_{Arg}	Dußle et al. 2004, SSR LG 1	Dußle et al. 2004; Wieckhorst et al. 2010
Pl_{13}	Mulpuri et al. 2009, SSR LG 1	Mulpuri et al. 2009

Resistance gene analogs (RGAs) and candidates (RGCs)

- Similar to other species, sunflower has many genes that also encode nucleotide binding site (NBS)-leucine-rich repeat (LRR) protein homologs. Usually they are duplicated, evolutionarily diverse, and arranged in the genome as large multi-gene clusters.
- 18 RGAs have been cloned and sequenced. Based on the sequences of two RGAs, 14 STS markers were developed within the PI_5 / PI_8 locus (Radwan et al. 2003, 2004, 2005).
- 630 NBS-LRR homologs have been identified from common and wild sunflower species using the sunflower expressed sequence tag (EST) database mining and comparative genomics approaches (Radwan et al. 2008).
- 167 NBS-LRR loci have been mapped in 44 clusters or singletons. Many of them were tightly linked to previously mapped downy mildew, rust, and broomrape resistance genes (Radwan et al. 2008).

Objective

- Molecular mapping of the downy mildew resistance genes in HA-R4 to races 300, 770, and a new hot race 734 on a sunflower genetic map, using SSR and EST-SSR markers.

Materials and Methods

Plant materials

- Mapping population: an F_2 population with 169 individuals derived from the cross of HA-R4 x HA 821.

  HA-R4: derived from an Argentine open-pollinated variety, 'Saenz Pena 74-1-2', resistant to rust, verticillium wilt (*Verticilium dahliae* Klebahnis), and at least nine downy mildew races, including a new hot race 734 (Gulya 1985; Gulya personal communication).

  HA 821: susceptible to all known races of downy mildew (Roath et al. 1986).

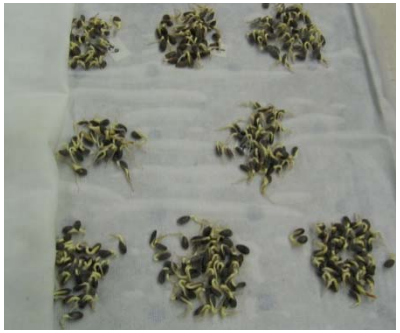
- Phenotype determination: testcross progeny (F_2 crossed to CMS HA 821).

- Another 37 germplasms or lines were used for the analysis of tightly linked markers.

Phenotype determination of F_2 individuals

Spore increase

Germination



Seedling selection

Inoculation

Grown in greenhouse for 11 days

Sporulation

Phenotyping

The whole seedling immersion technique according to Rahim et al. (2002), and the spore increase, germination, inoculation and sporulation are according to Mulpuri et al. (2009).

Polymorphic primer screening

- Bulked Segregant Analysis
- 246 pairs of SSR primers mapped to the 17 sunflower linkage groups
- Used additional 29 SSR and 26 EST-SSR markers on LG 1 and 32 SSR markers from LG 10 for further determination of the linkage group.

Statistical analysis and linkage map construction

- Chi-square test
- MAPMAKER/Exp version 3.0b program (Lander et al. 1987)
- Mapdraw V2.1 (Liu and Meng 2003).

Results and Discussion

1. Phenotype analysis in the population

Table 1. Segregation of the resistant to susceptible phenotypes in the population

Traits	Number of F_2 plants	Observed number[b]			Ratio tested	χ^2	P
		A	H	B			
Pl-300	169	41	87	41	1:2:1	0.15	0.929
Pl-770	167[a]	40	85	42	1:2:1	0.10	0.950
Pl-734	167[a]	39	85	43	1:2:1	0.25	0.884

The chi-squared test of the phenotypes of the F_2 population fit the Mendelian segregation ratio of 1:2:1 (χ^2 =0.15-0.25, P>0.05), indicating a single gene controlling the resistance.

a: No progeny test for two plants against downy mildew race 770 and 734.
b: Symbols: A (*PlPl*), H (*Plpl*), B (*plpl*), D (*PlPl* or *Plpl*).

Table 2. The two F_2 recombinants for the phenotypes among race 300, 770 and 734

F_2	Race 300	Race 770	Race 734
G96/1049	A	B	B
G96/1051	H	H	B

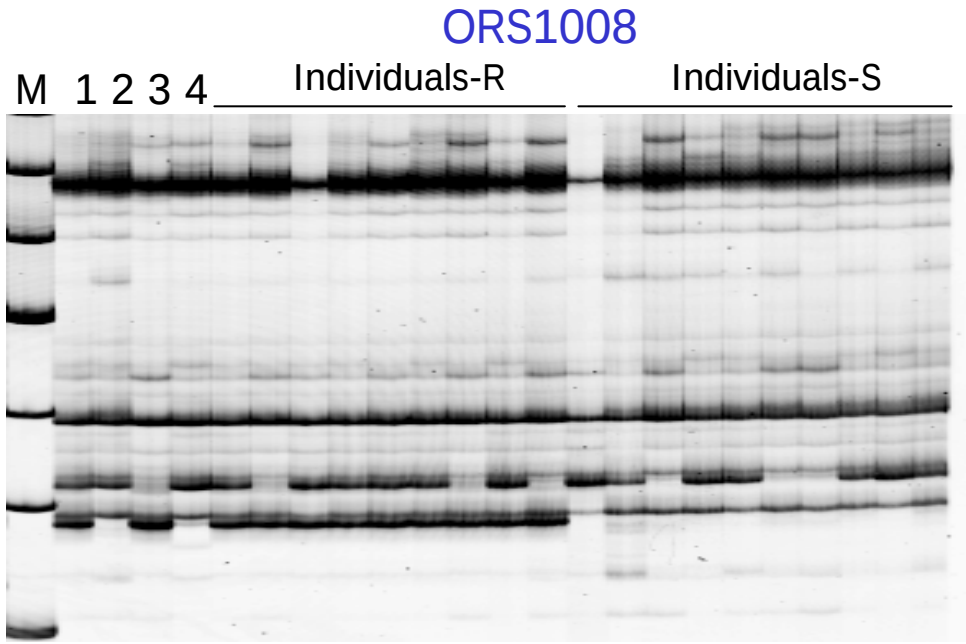
So far, the results suggest the existence of a tightly linked cluster of resistance genes in HA-R4.

2. Polymorphic marker screening and confirmation

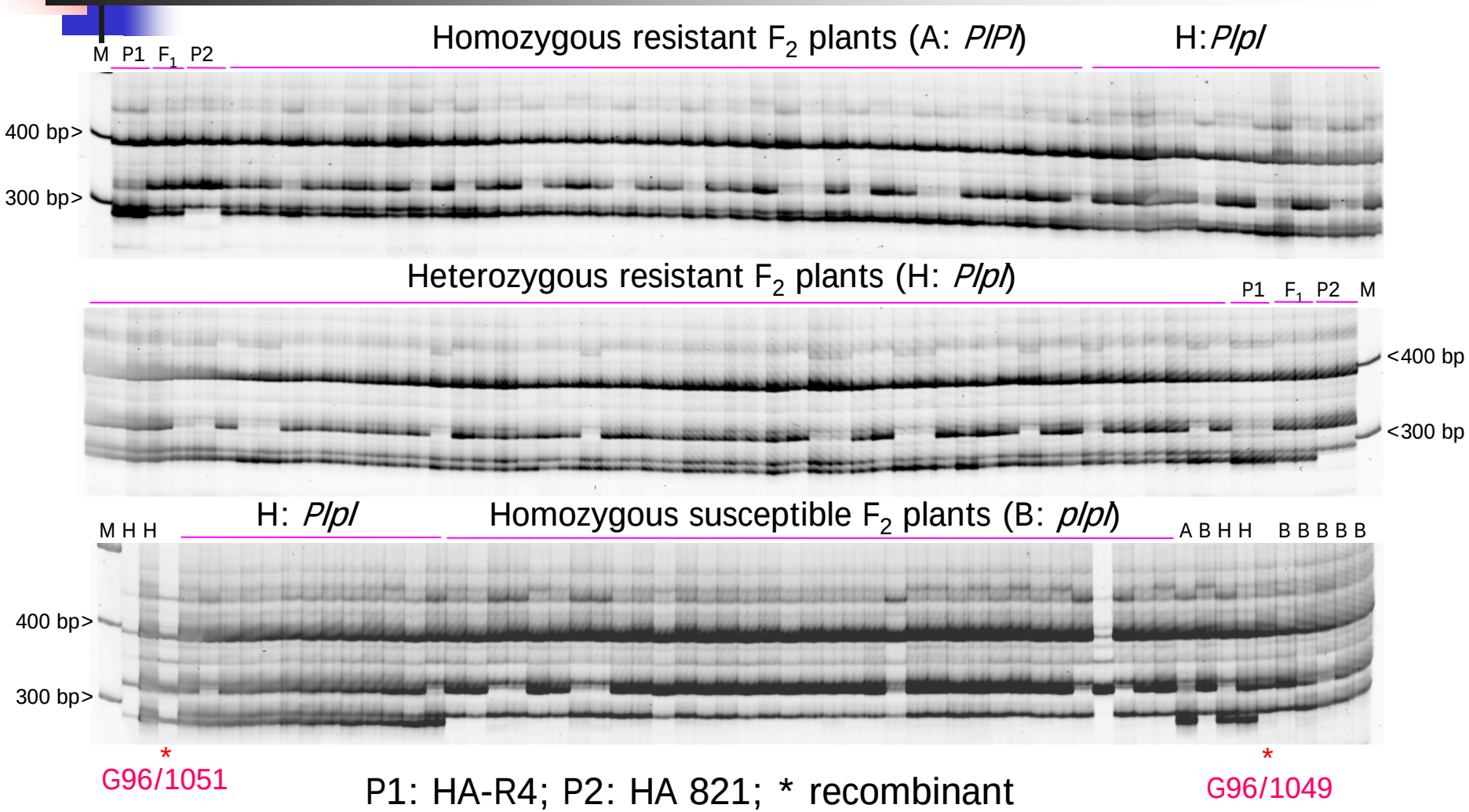
Among the 246 pairs of SSR primers:

Seven primers were polymorphic between the bulks (2.8%)

1. Resistant bulk
2. Susceptible bulk
3. HA-R4
4. HA 821
5. M: 100 bp plus ladder Gelpilot (Qiagen)



Amplification of ORS1008 in the F_2 population derived from HA-R4 x HA 821



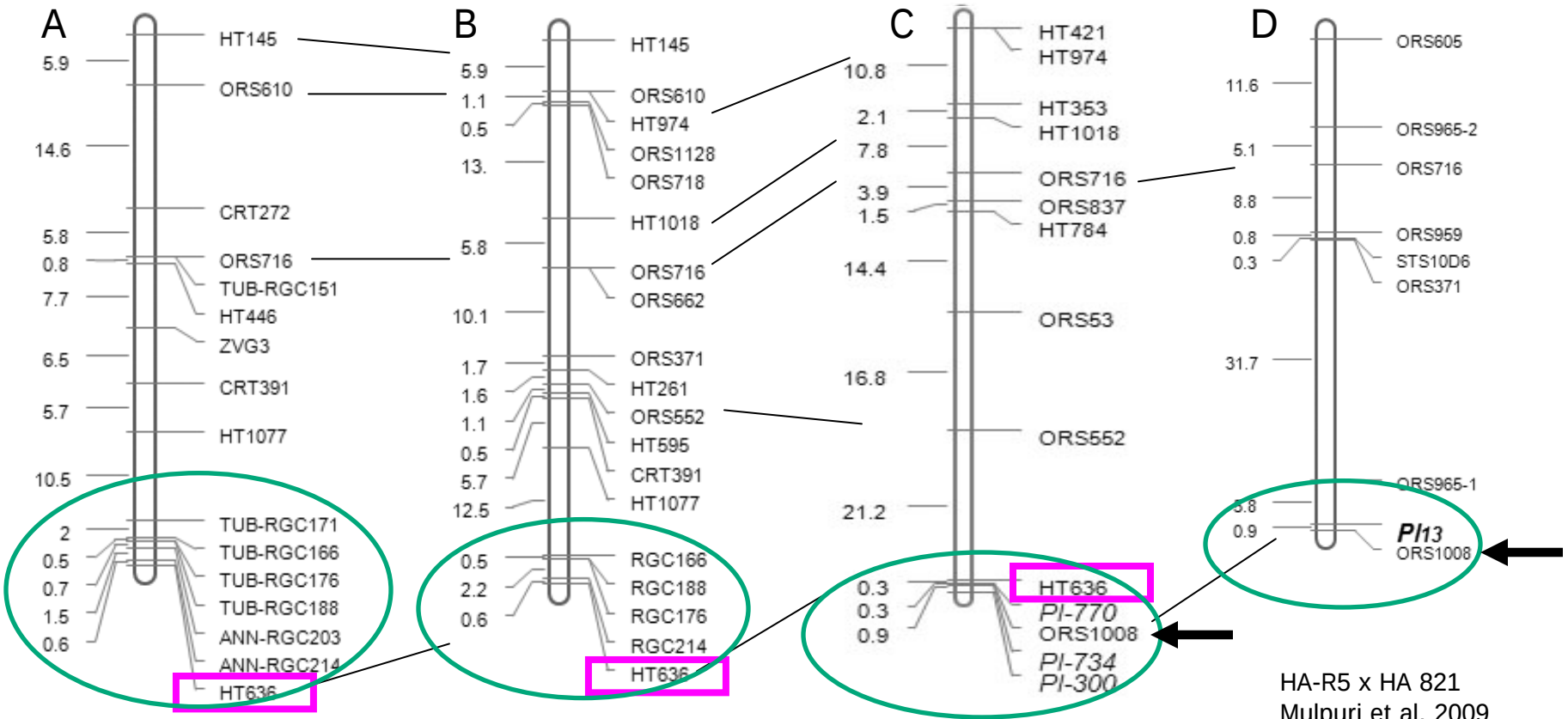
P1: HA-R4; P2: HA 821; * recombinant

Table 3. Segregation of the three downy mildew resistance (*Pl*) loci and 11 markers linked to *Pl* genes in the F_2 population of the cross HA-R4 x HA 821

Traits or markers	Number of F_2 plants	Observed number[b]				Ratio tested	χ^2	P
		A	H	B	D			
Pl-300	169	41	87	41		1:2:1	0.15	0.929
Pl-770	167[a]	40	85	42		1:2:1	0.10	0.950
Pl-734	167[a]	39	85	43		1:2:1	0.25	0.884
HT421	169	30	91	48		1:2:1	4.83	0.089
HT974	169	30	91	48		1:2:1	4.83	0.089
HT353	169	30	89	50		1:2:1	5.21	0.074
HT1018	169	30	88	51		1:2:1	5.51	0.064
ORS716	169	30	91	48		1:2:1	4.83	0.089
ORS837	169	32	88	49		1:2:1	3.71	0.156
HT784	169	32	83	54		1:2:1	5.78	0.056
ORS53	169	33	87	49		1:2:1	3.18	0.204
ORS552	169	33	85	51		1:2:1	3.84	0.147
HT636	169	40	88	41		1:2:1	0.30	0.860
ORS1008	169			42	127	1:3	0.00	1.000

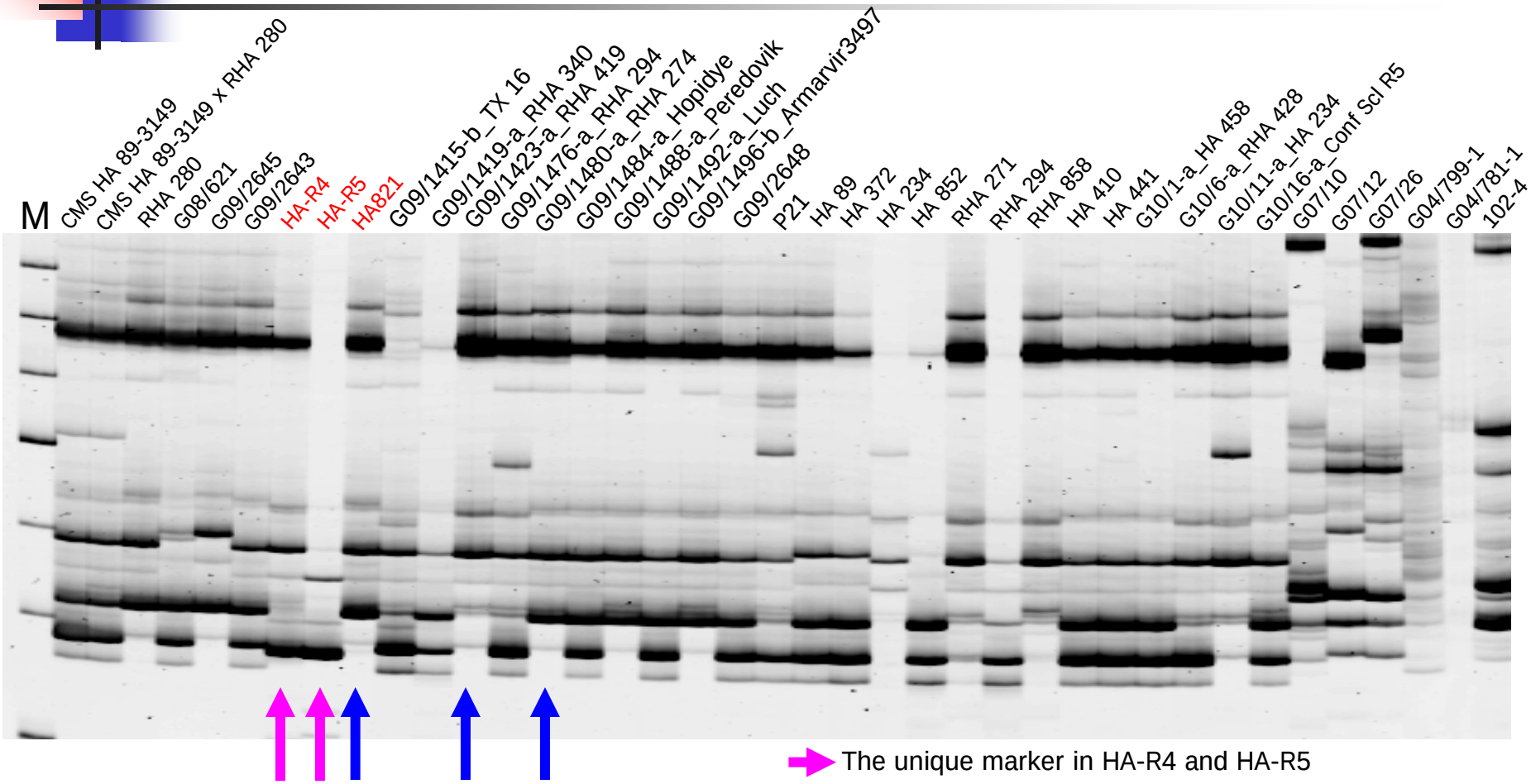
a: No progeny test for two plants against downy mildew race 770 and 734.
b: Symbols: A (*PlPl*), H (*Plpl*), B (*plpl*), D (*PlPl* or *Plpl*).

3. Linkage group construction



Is there any allelic relationship between the *PI* genes in HA-R4 and HA-R5?

4. Amplification of ORS1008 with 39 germplasms and lines



The *PI* genes in HA-R4 and HA-R5 are probably different than other downy mildew R materials tested.

Summary

1. Two recombinants were detected in the 169 F_2 individuals for downy mildew races 300, 770 and 734, suggesting a resistance gene cluster in HA-R4. It is mapped on LG 1 of the sunflower SSR map. Eleven markers are linked to this gene cluster, covering a genetic distance of 80 cM.
2. Two tightly linked markers ORS1008 and HT636 were identified, which will be useful for MAS.
3. ORS1008 and HT636 markers seem to indicate that the *Pl* genes in HA-R4 and HA-R5 are different from other downy mildew resistant materials tested.
4. We propose that there may be an allelic relationship of the *Pl* genes between HA-R4 and HA-R5.
5. Further determination of their relationship will be done using an allelic test.

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Questions or suggestions?